Characterization of the repetitive sequences in a 200-kb region around the rice *waxy* locus: diversity of transposable elements and presence of veiled repetitive sequences

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Repetitive genomic sequences might have various structural features and properties distinct from those of the known transposable elements (TE). Here, the content and properties of the repetitive sequences present in a 200-kb region around the rice *waxy* locus were analyzed using the available rice genomic database. In our previous Southern blotting analysis, 70% of the segments in this region showed smeared patterns, but according to the present database analysis, the proportion of repetitive sequences in this region was only 15%. The repetitive segments in this 200-kb region comprised 75 repetitive sequences that we classified into 46 subfamilies: 21 subfamilies were known TEs or repetitive sequences and 25 subfamilies consisted of newly identified TEs or novel types of repetitive sequences. The region contains no long terminal repeat (LTR) retrotransposable elements, but miniature inverted repeat transposable elements (MITEs) constituted a major class among the elements identified. These MITEs showed remarkable structural divergence: 12 elements were found to be new members of known MITE superfamilies, while five elements had novel terminal structures, and did not belong to any known TE families. Interestingly, about 10% of the repetitive sequences, including virus-like sequences did not have any of the usual characteristics of TEs, suggesting that a certain proportion of repetitive sequences that might not share the transpositional mechanisms of known elements are dispersed in the compact rice genome.

INTRODUCTION

Interspersed repetitive DNA is an intrinsic component of the fundamental frame of the genome architecture of eukaryotes (Britten and Kohne, 1968). In many cases, repetitive sequences (RSs) are referred to as transposable elements (TEs), which are usually categorized into two classes: class I, retroelements, which transpose replicatively by means of an RNA intermediate, and class II, nonreplicative elements, of which the DNA transposes in the cut-and-paste fashion (Charlesworth et al., 1995; McDonald, 1993). Although such representative TEs have been intensively mined from the genome databases based on sequence homology, and studied major players in the expression repetitiveness (Bureau et al., 1996; Jurka, 1998), few attempts have been made to isolate the

entire range of RSs distributed in a genome or a portion of a genome.

The genomes of various grass-species have been dissected to elucidate the genome organization and function with respect to the C value paradox proposed by Thomas (1971), i. e., the number of genes in different species is basically similar, while the genome sizes vary greatly. The rice genome is the smallest in size (about 430 Mb) among the genomes of grass species that have been characterized (Arumuganathan and Earle, 1991). Compared with maize and barley, which possess larger genomes, rice has a lower frequency of long terminal repeat retrotransposons (LTR elements) in its genome (Bennetzen, 2000). In the 340-kb *Adh1-Adh2* region of the rice genome, seven LTR elements (corresponding to 14% of this region) were found (Tarchini et al., 2000), whereas in the homeologous region of maize, the proportion of the LTR elements was much higher (74%) (San-Miguel et al., 1996). Similarly, the size difference

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between the homeologous sequences in rice chromosome 3 and barley chromosome 5H is explained by the proportion of LTR elements (Dubcovsky et al., 2001). The difference of the proportions of LTR elements between rice and these two crops accounts for the C value paradox in these grass species.

Despite the smaller number of LTR elements in the rice genome, Southern hybridization has shown that various rice genomic probes produce smeared patterns, which implies the presence of RSs. In fact, we previously reported that the use of probes from about 70% of the 260 kb surrounding the rice *waxy* (*wx*) locus resulted in smeared patterns in Southern blot analysis, which might have been due to repetitions of TEs or of novel repeated sequences (Nagano et al., 1999; Nagano et al., 2000). Two possible reasons for why the segments were recognized as smeared patterns in the blots were postulated: one is that

long RSs are located in the segments used as probes, and the second is that the restriction fragments to be detected were masked by highly repetitive short sequences contained in the probe segments. It is necessary to divide the segments more finely to distinguish between these possibilities.

In this study, we explored the sequences that produce the smeared hybridization patterns observed when probing with the segments around the rice *wx* locus. Detailed analysis of these repetitive sequences was made performed using the sequence information available in the rice genome database. We identified 75 RSs in the 200-kb region around the *wx* locus. Among these 75 RSs, there were no LTR elements, and most of the RSs were short (less than 1000 bp). Our survey of the region around *wx* suggested that the rice genome contains numerous kinds of RSs that include not only a large

Fig. 1. Local genome map for repetitive sequences and genes in the 195-kb region around the *wx* locus of rice chromosome 6. The purple histogram shows the blast-hit number every 500 bp. These are the number of homologous locations resulting from a blast search when a 500-bp segment was used as the query (version 2.0, <http://www.ncbi.nlm.nih.gov/blast/>) against the rice genome sequence. The red bars below the histogram indicate repetitive sequences identified by the database survey, and the numbers (RS no.) of the bars correspond to those of the element listed in Table 1. Arrows indicate the direction of transcription of a gene, and blue, green and yellow bars show open reading frames of hypothetical genes, unknown genes and known genes, respectively. The gray bars indicate the locations of hybridization probes used to determine the actual copy numbers as shown in Fig. 2.

number of miniature inverted repeat transposable elements (MITEs) (Wessler et al., 1995), but also a certain proportion of hitherto unknown new elements without any features of ordinary TEs.

MATERIALS AND METHODS

RS site detection and mining TEs The sequence of the 195 kb around the *wx* locus was obtained from two rice genomic clones (Accession nos. AP000559 and AP002542) located on the short arm of chromosome 6. The 195-kb sequence was divided into 390 segments of 500 bp each, and each segment was subjected to blast search queries (version 2.0, <http://www.ncbi.nlm.nih.gov/blast/>) against the rice genome sequences that had been registered in DDBJ (<http://www.ddbj.nig.ac.jp/>) as of November 2001, when the sequence of a quarter of the total length of the rice genome had been registered. The numbers of the rice genome sequences that shared similarity (blast score >80) with the query sequence were counted, and the number of locations homologous to each query segment was plotted. To establish a standard for RSs, Southern blotting analysis was performed using as probes several rice-genome segments with different re-

iteration frequencies. The segments which were found to have over 10 repetitions were judged to be RS sites, because the Southern blotting of such segments resulted in smeared patterns (Fig. 2). To characterize RSs, we first looked for the end points of the RSs in multiple alignments of homologous sequences. Secondly, blast searches were performed to identify homologies with known TEs. We then attempted to find LTR or TIRs which might have flanked RSs in either the original query of the *wx*-proximal or the other homologous genomic regions. For RSs that did not have any characteristics of known TEs, the rice genome database was used to perform a search for related-empty site (RESite) (Le et al., 2000), which provides evidence for the transposition of RSs in the form of the empty donor site.

Southern hybridization Rice (var. Shimokita) genomic DNA was digested with *Hind*III, electrophoresed in 0.7% agarose gels, transferred to nylon membranes (Pall: Bio-dyne B), and hybridized. About 0.1 ug of each of the indicated subcloned fragments or PCR fragments (Fig. 2) was prepared as an ECL probe. The membranes were washed twice after hybridization under the standard ECL conditions with 0.5 M NaCl at 42°C for 20 min and

Fig. 2. Southern blot analysis to examine copy numbers at several locations with different reiteration frequencies.

Probes were prepared from six locations, as indicated in Fig. 1 (gray bars: a-f). The rice genomic DNA (var. Shimokita) was digested with *Hind*III and electrophoresed in 0.7% agarose gels. The reiteration frequencies of regions in the rice genome used as probes were determined by blast search and are indicated below the blots.

Table 1. A list of repetitive sequences found in the 195-kb region around the *wx* locus and the classification as to TEs.

Class	Family	Subfamily	RS no. (Position) ^a	Reference
I	<i>p-SINE</i>	<i>p-SINE1</i>	41(B: 90136-90259), 42(B: 93272-93396), 64(B: 124352-124461)	Umeda et al. (1991)
II	<i>CACTA-like</i>	<i>CACTA-like</i>	48(B: 99148-100167)	Tarchini et al. (2000)
	<i>Tourist</i>	<i>Castaway</i>	63(B: 123684-123948)	Bureau et al. (1996)
		<i>Ditto/Type F</i>	16(A: 84793-85042), 27(A: 121759-122008)	Bureau et al. (1996)
		<i>Gaijin</i>	56(B: 112132-112282)	Bureau et al. (1996)
		<i>Type C</i>	12(A: 80473-80735), 34(B: 72197-72530), 37(B: 78729-79073)	Tarchini et al. (2000)
			52(B: 106052-106315), 57(B: 112314-112654), 74(B: 155068-155319)	
		<i>Type E</i>	59(B: 117784-117935)	Tarchini et al. (2000)
		<i>Tabito I</i>	1(A: 44224-44419), 2(A: 46779-47024), 68(A: 131694-131892)	This study
		<i>Tabito II</i>	3(A: 48815-48961), 7(A: 68719-68871), 9(A: 74458-74609)	This study
		<i>Tabito III</i>	5(A: 62279-62406)	This study
		<i>Tabito IV</i>	10(A: 75414-75763)	This study
		<i>Tabito V</i>	30(A: 133780-134025)	This study
		<i>Tabito VI</i>	58(B: 115872-116008)	This study
	<i>Tnr1/Stowaway</i>	<i>Tnr1</i>	25(A: 120553-120778), 35(B: 73010-73256), 43(B: 94109-94346)	Umeda et al. (1991)
		<i>Stowaway-Os4</i>	44(B: 95126-95334)	Bureau et al. (1994a)
		<i>Stowaway-Os6</i>	70(B: 137134-137290)	Bureau et al. (1994a)
		<i>Type A</i>	20(A: 98815-99051), 69(A: 136718-136965)	Tarchini et al. (2000)
		<i>Type B</i>	14(A: 83018-83258), 21(A: 109869-110107), 45(B: 95600-95843)	Tarchini et al. (2000)
			61(B: 122424-122661), 65(B: 124952-125186), 66(B: 127346-127569)	
		<i>Kiseru I</i>	17(A: 90206-90432)	This study
		<i>Kiseru II</i>	29(A: 123860-124122)	This study
		<i>Kiseru III</i>	38(B: 81992-82213)	This study
		<i>Kiseru IV</i>	47(B: 98292-98457)	This study
	<i>Mu-like</i>	<i>Mermite</i>	54(B: 110574-110973)	Tarchini et al. (2000)
		<i>Onaga I</i>	19(A: 96437-96575), 39(B: 85285-86938), 40(B: 86993-88410)	This study
			50(B: 103062-103426)	
		<i>Onaga II</i>	53(B: 107036-107754)	This study
	<i>Akan</i>	<i>Akan</i>	18(A: 91324-91919)	This study
	<i>Mashu</i>	<i>Mashu</i>	26(A: 120822-121088), 75(B: 155536-155804)	This study
	<i>Saroma</i>	<i>Saroma</i>	33(A: 137816-138389)	This study
	<i>Shikotsu</i>	<i>Shikotsu</i>	55(B: 111333-111487), 67(B: 128728-128985)	This study
	<i>Toya</i>	<i>Toya</i>	62(B: 123362-123471)	This study
Unknown	<i>Basho</i>	<i>Basho</i>	4(A: 57083-58173), 46(B: 96817-98291), 49(B: 100171-100705)	Turcotte et al. (2001)
			60(B: 120882-121251)	
	<i>Explorer</i>	<i>Explorer</i>	28(A: 123621-123805)	Bureau et al. (1996)
	<i>Wanderer</i>	<i>Wanderer</i>	8(A: 69603-69824)	Bureau et al. (1996)
	<i>Type Unique</i>	<i>Type Unique</i>	32(A: 136026-136128), 36(B: 75964-76185)	Tarchini et al. (2000)
	<i>Moiwa</i>	<i>Moiwa</i>	73(B: 154334-154487)	This study
	<i>Rishiri</i>	<i>Rishiri</i>	71(B: 139698-139937)	This study
	<i>Shari</i>	<i>Shari</i>	51(B: 103834-104257)	This study
	<i>Taisetsu</i>	<i>Taisetsu</i>	31(A: 135339-135595)	This study
	<i>Teine</i>	<i>Teine</i>	6(A: 65425-65665)	This study
	<i>Tengu</i>	<i>Tengu</i>	15(A: 84494-84563)	This study
	<i>Youtei</i>	<i>Youtei</i>	72(B: 153655-153790)	This study
	<i>RTBV-like</i>	<i>RTBV-like</i>	23(A: 112058-119385)	Nagano et al. (2000)
	<i>Microsatellite</i>	MRG6379	11(A: 79154-79181)	AY024054
		MRG0415	13(A: 81499-81522)	AYO18090
		(AT) _n	22(A: 111644-111987)	This study
		(AT or AC) _{x124}	24(A: 119462-119713)	This study

^a: RS (repetitive sequence) no. is indicated below each red box in Fig. 1. Numbers in parentheses show the nucleotide position in Accession no. registered in AP000559(A) or AP002542(B).

detected using the ECL gene detection system (Amersham).

Accession numbers of TEs identified in this study

Each consensus sequence of the TEs identified in this study was surveyed by use of the rice genome database. Use of these consensus sequences readily identified the counterparts from the rice genome database. Thus, we registered these representatives to DDBJ, and the nucleotide sequences of the TEs and their accession numbers appear in Nucleotide Sequence Databases as follows: TabitoI: AB077830; TabitoII: AB077831; TabitoIII: AB078024; TabitoIV: AB078025; TabitoV: AB077832; TabitoVI: AB077833; KiseruI: AB077834; KiseruII: AB077835; KiseruIII: AB077836; KiseruIV: AB077837; Akan: AB077838; Mashu: AB077839; Saroma: AB077840; Shikotsu: AB077841; Toya: AB077842; Moiwa: AB077843; Rishiri: AB077844; Shari: AB077845; Taisetsu: AB077846; Teine: AB077847; Tengu: AB077848; Youtei: AB077849.

RESULTS

Search for RSs in the 200 kb around *wx* of the rice genome database The rice genome database is now available for 195 kb of the 260-kb region surrounding the rice *wx* locus examined here (as of November 2001). In this 195-kb region, the rice genome project has identified 41 putative genes, including five unknown and 24 hypothetical genes (Fig. 1). The average sizes of the genes and spacers in this region are 2.02 and 2.87 kb,

respectively. We divided the 200-kb region into 390 segments (each 500 bp in length), and subjected these segments as blast search queries against the rice genome sequences registered in the DNA database. Sequences at different locations of the rice genome that hit the query (score over 80) were counted, and the blast-hit number was regarded as the reiteration frequency of each segment, as summarized in Fig. 1.

To examine whether the repetition of the segments used as queries reflects the actual copy number in the genome, we carried out Southern blot analysis using some representative segments as probes (Fig. 2). The segments determined by blast search to have single or low copy number in the genome showed a single or a few hybridized bands in Southern blotting (Fig. 2b, c, f). In the blotting pattern of a segment with 10 repetitions, about 20 bands were observed in the blot (Fig. 2d), and more highly repeated segments produced more bands or smeared patterns (Fig. 2a, e). These blotting results indicated that the repetitions recorded by blast analysis are mostly underscores, but are roughly correlated with the actual copy number. Of a total of 390 segments, 259 did not have similarity with other rice genomic regions. When sites that were scored as having less than 10 repetitions were classified as non-RSs, about 77% of total sites were included in this category. Segments with more than 50 reiterations were found in only 49 segments. Therefore, our previous finding by Southern-blot analysis that 70% of the 200-kb around the *wx* locus consisted of repetitive regions (Nagano et al., 1999) was an apparent overestimation that was caused by abundant

Table 2. Categorization of TE subfamilies into Tourist, Tnr1/Stowaway and Mu-like families according to their TIRs and TSDs.

Family	Subfamily	Consensus sequence of TIR ^a	Maximum length of TIR (bp)	Length of TSD (bp)	Reference
Tourist	<i>TouristI</i>	GGGCCAGTTAGTT	14	3	Turcotte et al. (2001)
	<i>Castaway</i>	GGCCCRRTTGA	12	3	Bureau et al. (1996)
	<i>Ditto/TypeF</i>	CAGCARGWYAAATAG	15	3	Bureau et al. (1996)
	<i>Gaijin</i>	GGCTCTGTTAG	12	3	Bureau et al. (1996)
	<i>TypeC</i>	GGSTGTGTTAG	12	3	Tarchini et al. (2000)
	<i>TypeE</i>	GSSYKWTGTTAGTTCC	16	3	Tarchini et al. (2000)
	<i>TabitoI</i>	GGRYVGGTTTTRGTTTGWGRYCTAAATWRGM...	44	3	This study
	<i>TabitoII</i>	SGSYGTGTTAGTTCC	15	3	This study
	<i>TabitoIII</i>	RGCTTGTTGKTT	14	3	This study
	<i>TabitoIV</i>	GGGSGTGTTRGWNKCCAGGGCTAAAATTT...	160	3	This study
	<i>TabitoV</i>	GGCTSTGTT	9	3	This study
	<i>TabitoVI</i>	GGCTCTGTTAG	12	3	This study
	Common	GGSYSTGTTTRGTT			
Tnr1/Stowaway	<i>Tnr1</i>	CTCCCTCCGTTTCTRAATATTCRCGGTTRT...	75	2	Umeda et al. (1991)
	<i>Stowaway-Os1</i>	CTTCCTCCGTTTCCACATGTAAGTCATTCT...	70	2	Bureau et al. (1994a)
	<i>Stowaway-Os4</i>	CTCCCTCCGTTTCATTTTAAGTCAACTAT...	36	2	Bureau et al. (1994a)
	<i>TypeA</i>	CTCCCTCCRTTTCATATTAATATYGTGTTG...	37	2	Tarchini et al. (2000)
	<i>TypeB</i>	CTACCTCCRTTWTTTAATAKATGACGCCGT...	70	2	Tarchini et al. (2000)
	<i>KiseruI</i>	CTCCCTCCRTACTCRTAAAGRAATCGTTT...	36	2	This study
	<i>KiseruII</i>	CTCCCTCCGTTCCMAAATATAAATCAATTTT...	31	2	This study
	<i>KiseruIII</i>	CTYCTCTCATCCYATAATATAAGCGY	27	2	This study
	<i>KiseruIV</i>	CTCCCTCCGTTCCCAVAATATAAGGGATTTT...	78	2	This study
	Common	CTCCCTCCRTYYYATAATATAAR			
Mu-like	<i>RMu-A23</i>	GAGAAATTCGTGATTTTGCTATCCGAAAG...	193	9	AB023047
	<i>Mermite</i>	GGAATAAGTTCACCTTCCTGACCCCTCARWGT...	177	9	Tarchini et al. (2000)
	<i>Onagal</i>	GCAAWAAGTWYAYCKRAGTCCCTTTRACTT...	297	9	This study
	<i>OnagaII</i>	GAGTAAAGTCCATCACCGGTCCCTAAACTT...	383	9	This study
	Common	GRRAWAAGTTYAYYTCNGGTCCTCAACTT			

a: a consensus TIR motif of each TE subfamily was obtained by comparisons of at least 5 elements. Letters in black shadows show the consensus sequences of the TE families, letters in gray shadows contain consensus nucleotides.

small RSs (mostly <500 bp) distributed in this region.

Characterization of the RSs To identify the repeated sequences in the 200-kb region, we performed blast analysis on 88 segments from this region that were reiterated more than 10 times in the rice genome (Fig. 1). The results obtained are summarized in Table 1 and Fig. 1. In the 88 segments, 41 RSs were identified as previously described TEs, including class I and class II TEs and TEs not included in these classes. The class I elements in the region comprises only pSINE sequences found at three sites (Mochizuki et al., 1992; Mochizuki et al., 1993; Hirano et al., 1994), and no LTR elements were identified. The blast analysis identified 26 RSs homologous to known class II elements, and these RSs fell into 12 distinct subfamilies: 10 subfamilies were classified as known MITEs (Tourist and Tnr1/Stowaway family),

which are short sequences (<500 bp) with conserved TIRs and 2- or 3-bp target site duplications (TSDs) (Bureau and Wessler, 1992; Bureau and Wessler, 1994a; Bureau and Wessler, 1994b; Bureau et al., 1996; Tarchini et al., 2000), the rest were found to be similar to CACTA-like elements (Tarchini et al., 2000) or snapback elements (such as Mermite) (Tarchini et al., 2000). Eight of the RSs fell into neither of these TE classes, and contained TIRs and/or TSDs that have not been identified. These eight RSs were divided into four types of TEs with strong similarity (> 80%) to Explorer (Bureau et al., 1996), Wanderer (Bureau et al., 1996), Type Unique (Tarchini et al., 2000) or Basho (Le et al., 2000; Turcotte et al., 2001), respectively. The rice Basho element, which carries minisatellite-like sequences, was found at four sites (Inukai and Sano, 2002). Basho is composed of repeats of a 78-bp unit sequence, with unit number varying from one

TIR 284bp / TSD 3bp

TIR 11bp / TSD 8bp

TIR 10bp / TSD 8bp

Fig. 5. The new families of class II elements identified in the 100 kb region around *swt1*. Five new members of each family were identified in the rice genome accessions denoted on the left side, and their positions are noted at the end of the alignment. The black shadows in the alignments show consensus sequences of the family members. The arrows indicate TIRs and the gray shadows represent TSDs.

to six among the elements. Four microsatellite sequences, two of which were deposited in the database, were found in this 200-kb region.

The remaining 34 RSs were unidentified elements, each of which has highly homologous copies (>80%) located in other genomic segments. A computer-aided search indicated that 26 RSs have TIR or TIR-like sequences, but seven RSs have no detectable characteristics of known TEs or characteristics of RSs such as microsatellites. Curiously, some continuous segments (RS no. 23) located about 50 kb upstream of *wx* showed similarity to the rice tungro bacilliform virus (RTBV), which is a pararetrovirus. Recent reports suggest that plant pararetrovirus DNA might integrate rather frequently into host chromosomes (Jakowitsch et al., 1999; Harper et al. 1999; Ndownora et al., 1999).

Classification of TE-like sequences Of a total of 75 RSs found in this study, we accounted for 37 RSs as known TEs, while 26 newly discovered RSs are possible class II elements, because they possess TIRs. The entire sequences of these 26 RSs have little similarity to the sequences of previously reported TEs. However, classification of TEs has usually been based on TIR and target site duplication (TSD) sequences. Hence, we compared the TIR structures with those of known elements. Turcotte et al. (2001) reported that the rice TEs were categorized into three major groups: Tnr1/Stowaway, Tourist and Mu-like families. Using their classification system based on the identities of TIR and TSD, 19 RSs found in this study were categorized into the three TE families, and their subfamilies were sorted out by the entire similarity (> 80%) in addition to TIR and TSD as follows:

Tourist family: *Tourist* was originally discovered in the maize *wx-B2* allele (Bureau and Wessler, 1992), and Bureau and Wessler (1994) also identified Tourist-like elements in the rice genome. These elements have a consensus TIR sequence, GGGCCAGTTTAGTT, with a three-nucleotide (TTA) TSD (Table 2). Other elements in this category, Castaway (Bureau et al. 1996), Ditto/MITE-Type F (Bureau et al., 1996; Tarchini et al., 2000), Gaijin (Bureau et al., 1996), MITE-Type C and E (Tarchini et al., 2000) possess similar terminal sequences, as shown in Table 2. Furthermore, we identified 10 RSs which we classified into the Tabito (Tourist in Japanese) I-VI subfamilies, which also harbor Tourist-like terminal sequences, and have flanking 3-bp TSDs (Table 2). A total of 21 RSs in the 200-kb region examined appear to be classifiable into the Tourist family. The common TIR motif of the Tourist family was identified as GGSYST-GTTTRGTT.

Tnr1/Stowaway family: Tnr1 was first discovered in an intron of the rice *wx* gene by Umeda et al. (1991), and subsequently Bureau and Wessler (1994) independently identified Stowaway in a wide range of plant species. These

two types of elements are structurally similar, and here we refer to them as Tnr1/Stowaway. Among the unclassified RSs in this study, the ends of the Kiseru (Stowaway in Japanese) I-IV sequences shared a common TIR motif with the ends of the Tnr1/Stowaway elements, although the other structures and the sizes of the TIRs varied among the elements (Table 2). In addition, the MITE-Type A and B elements described by Tarchini et al. (2000) also appear to have a TIR motif analogous to that of Tnr1/Stowaway (Table 2). Thus, a total of 17 elements possibly belonging to the Tnr1/Stowaway family were found in the 200-kb region. The common TIR motif of the Tnr1/Stowaway family is CTCCCTCCRTYYYATAATATAAR, and TA is a typical preferred target site for these elements (Table 2).

Mu-like family: According to Yu et al. (2000), Mu-like elements contain long TIRs (>100 bp) with a 10-bp consensus motif (GGGGAAAAAT) at the termini and 9-bp TSDs. Mermite (Tarchini et al., 2000), which possesses GA-rich ends and TIRs longer than 160 bp and generates a 9-bp TSD, shows Mu-like characteristics (Table 2). Mermite forms a snapback structure. The six Mu-like RSs found in the 200-kb region share these common features: long TIRs with GA-rich ends, 9-bp TSDs and snapback structure (Table 2). Their TIR sequences are also quite similar to those of Mermite, and were analyzed to determine a consensus motif (Table 2). The elements were divided into two subfamilies, named Onaga ("long tail" in Japanese) I and II. We judged that these six RSs, like Mermite, belong to a Mu-like family.

Identification of new TEs Fourteen RSs did not match any of the known TEs. These unidentified sequences included seven possible TEs, whose ends contained TIRs. The seven RSs were classified into five elements: Akan, Mashu, Saroma, Shikotsu and Toya. Mashu and Shikotsu appeared at two sites in this 200-kb region. Each of these five elements is quite conserved in the rice genome, as shown in Fig. 3A–E. The individual elements contain distinct TIR sequences. Saroma has remarkably long TIRs (200–284 bp) that form snapback structures (Fig. 3C). The 3-bp TSD of this element is different from that of Mu-like elements, which contain 9-bp TSDs. We could not find complete TIRs for Akan and Toya in the 200-kb region, but we discovered their TIRs in other locations (Fig. 3A and E). These newly identified elements are reiterated at low frequency as shown by the blast search, and the entire structures of the element are relatively less divergent within a given family compared with the structure of the Tnr1/Stowaway, Tourist and Mu-like families (data not shown). This suggests that their invasion into the rice genome occurred relatively recently.

The sequences of seven RSs, namely Moiwa, Rishiri, Shari, Taisetsu, Teine, Tengu and Yutei, could not be

Table 3. Conservation of sequences that were identified as possible TE elements in the rice genome but which do not have typical TE characteristics.

Name of unknown RS	Sequence found in the region around <i>wx</i>	Similar sequences found in the other regions ^a	Identity (%) ^b
<i>Moiwa</i>	TAAAAGGTGGCGGGCTCTAATCCATTGGACTAGTCTTTTGGGCTGTGTAGGCTCAGGA CCTAAAGTTTGGTCTCGTTAGGCTTGTGGTGAAGCAGGCCAATGTGATCTGGGTGAC CCATAGTTGGTGGACAGGTTGCGCACTCTAACA	AC079890 (32920 -33074) AC091732 (81826 -81980) AJ307662 (26999 -27151) AP001550 (87368 -87520) AP002485 (98511 -98661)	89.0 87.7 86.0 86.0 81.8
<i>Rishiri</i>	TTATCTATATCATTATAAAAAATTGAAGATGTTTTTACCGGTATTTTGTACTCATTGTG CATGAGTCGGTTTTTAAGTTTGTTCGCTTTTGGAAATACATATCCGTATTTGAGTAGTGT TTTAAGTTCGTTTCGTTTTTTGAAATACAAAAGGAATCGTAAATAAATCTATTTTAAAAA ACTCGCATGCTAAGTTGAGCATCGAATGCTAATTCAGCTCATAATTTTCCAAAAA	AP000969 (76848 -77086) AF172282 (300200-300436) AC079887 (69157 -69399) AP003199 (108439-108676) AP003022 (99665 -99906)	80.4 80.0 79.2 77.9 75.8
<i>Shari</i>	GTGCAAAACCATTAACTATTTTTTCTCTTAAATATGTATCCAAATGATGATCCGA TTGCACCATTAATTCGTGCAATTAAATCTTTAAACAATACCACACATGGATATATTT CGACGAAAAAATAGCTTATTATGAAATTATTTTAAATGTTGCACGATGTTCCACCAAG TATATTGGAATTGTTTTAAATAAGTAGATTGAAATGTTCAATCGTTTAAATCGGGCGTT GTTTCACCTTAAATAAAACAATGTTTCAGCAATAACGAAAGAAATGTTCTATTTCATTG CAACATTAGATCTATACATAGTGAACATTATGAGTACACTAGGTGAACATTTTGGTG AACAAAGAAGCAATTCCTTAATAGAGTCTTCTTAAATATGTGGATGATTGTTCCA AAG	AC034258 (55751 -56180) AP0675A05 (110035-110462) AP002899 (17588 -18015) AL442110 (103245-103631) AC079887 (167297-167724)	90.3 89.2 88.9 79.2 78.1
<i>Taisetsu</i>	AGGTAGTGTGGTGGCAAGGATGGGATAGAATGGGATGGGATGAACCCACTTTTAGGGG TGTTTGGTTGAGAGGCGAATGGGATGGGTGGTCCCTGGAGAGAAATATCCCTCAGAT CGGGACGAAGTGGTCCGCCAAAATCAGCCAGACCAATCCATCCACTTCGACCGAGCGA ACGGCGTCAGCT	AC068924 (144842-145033) AP003222 (7871 -8062) AP003282 (655791-65770) AP000391 (26620 -26810) AP003487 (29924 -30102)	91.1 90.6 88.0 84.4 81.3
<i>Teine</i>	GCGAAGTAAGCGTAGCTCATCTGGTTAGGTTCTTGTGGAGGAAGTCCACCTACATT CAAATCCTAAATTTGTTTCGCAATTTATGGCTAATATTCTTTTAAATGGTAGACGACGTACC CGTCGACAGCGAGAGCTTGGTGACTTCGTCAATCTCAGATTGTTGGTCCATCTCCG AAGAGACTCATATGGATAGGGGATA	AC026815 (82575 -82791) AP003046 (83906 -84122) AP002485 (91040 -91256) AP003210 (30114 -30345) AL442110 (85995- 86209)	79.5 76.1 75.6 73.7 72.2
<i>Tengu</i>	TTTGAACCAGAATAAAAAATATCTTTAGTCCCGGTTCTATTCGAACCAGGACTATTGT GGATTTTGGG	AP002817 (43658 -43727) AP002870 (58716 -58785) AC087544 (51651 -51720) AC073391 (141938-142007) AL442110 (12146 -12215)	88.6 87.1 87.1 87.1 77.1
<i>Youtei</i>	ACTAGCAATATTATCTAGAGTATTTTAAATATCATGCAACATGATATATCAATTACAA TTTTGTTGTTATTTTAGAATCAATATGCAACCAATGTCAATCATTATCTCCACTTCAT TTTCACACTATTTAAA	AP003296 (137134-137270) AP002953 (132484-132619) AC027660 (44135 -44271) AC073393 (106378-106511) AP003213 (122239-122374)	89.0 87.5 87.5 87.5 86.8

a: Accession no. and nucleotide positions of each similar sequence.

b: The identity between a repeated sequence found in the region around *wx* and a similar sequence in a different region was calculated.

easily classified as typical TE, since we failed to detect in them any characteristics of TEs. In contrast to the case of the RTBV-like sequence described above, no particular homology to any known sequence have been found in these RSs so far. It was also difficult to find the RESites (Le et al., 2000) for the empty donor sites of these elements in the rice genome database. In Table 3, we summarize the locations and homologies of these seven RSs and the corresponding sequences at five different locations. All of these sequences showed more than 70% identity with the similar sequences at the other locations. Like Basho (Le et al., 2000), Crackle (Turcotte et al., 2001), Explorer (Bureau et al., 1996), and Type Unique element (Tarchini et al., 2000), whose transposition mechanisms are unclear, the RSs found in this study may be novel types of TEs. It is notable that such novel RSs with yet unknown structures constituted 10% of the total RSs. This fact leads us to assume that numerous unknown RSs remain to be found.

DISCUSSION

This study examined the content and structures of the RSs present in the 200-kb region around the *wx* locus in the rice genome. We identified 75 RSs in this region and

classified them into 46 subfamilies, of which 23 were newly identified types of TEs or possible TEs. The average interval between RSs in the 200-kb region was estimated to be 2.7 kb, implying that there is on average one RS per spacer. In fact, 44 RSs were distributed in spacer regions, and 19 RSs resided in introns. Most of the RSs were short (<500 bp), and no long RSs (>5000 bp) such as LTR elements were observed in this region. Our results showed that these short elements possess various structural features, implying the presence of diverse and abundant class II elements in the rice genome. The properties of RSs detected in this local survey around *wx* are in accord with the findings of the overall genome survey of rice (Turcotte et al., 2001). Similarly, the compact genome of *Arabidopsis* tends to contain relatively few class I elements and an abundance of class II elements. In addition to these findings, we found a much larger number of novel TEs and unknown RSs than expected based on the overall genome surveys of rice (Turcotte et al., 2001) and *Arabidopsis* (Le et al., 2000): (proportion of unclassified elements: in the rice *wx* region: 17%; the overall rice genome: 3%; the overall *Arabidopsis* genome: 0.3%), suggesting that a large number of unknown elements remain to be mined.

We do not yet know whether all TEs in the same family

share a common transposase, or the different subfamilies of TEs utilize individual transposases, which would complicate the classification of TEs. Each subfamily of class II elements was found to contain unique TIRs, and even in the same TE family, the consensus TIR of each subfamily within the family was slightly different (shown in Fig. 3). These facts could be simply explained by the possibility of divergent transposases. Most of the elements detected thus far should lack the ability to transpose due to loss of the transposase. The divergent TIR sequences might reflect the evolutionary history of each TE family. Indeed, the TEs found in this study are differentially distributed in various *Oryza* species (Nagano, Kishima and Sano, unpublished data). Prior to the rice speciation, various class II elements must have invaded or been generated at different times and then evolved.

Deshpande and Ranjekar (1980) estimated by means of a Cot assay that the RS segments account for 50% of the content of rice genome. Recently, Tarchini et al. (2000) estimated the contribution of TEs to 340 kb of the rice *Adh1-Adh2* region in chromosome 11 as 28.5%. Turcotte et al. (2001) also surveyed 910 kb of the rice genomic DNA and found that TEs constituted 19.9% of the examined regions. In the present study, only 15% of the 200-kb *wx* region showed repetitive characteristics. Although the proportion of RSs in the region around *wx* might thus be lower than that in the other genomic regions noted above. TE- or TE-like sequences occur more frequently in the *wx* region than in the other reported regions: the average densities of TEs in the *wx* region, *Adh1-Adh2* region and 910 kb of rice genomic DNA can be calculated as one in every 2.95 kb, 3.51 kb and 4.5 kb, respectively. There are 41 annotated genes, including five unknown and 24 hypothetical genes in the 200-kb *wx* region around the rice *wx* gene. Hence, the average sizes of the genes and spacers in this region are 2.02 and 2.87 kb, respectively. Thus, one TE or TE-like element would be expected to be located in every spacer. On the other hand, the absence of LTR elements in this region probably accounts for the lower percentage of total length of RSs and the higher density of RSs. This particular set of properties might influence the recombination frequency. Inukai et al. (2000) analyzed the recombination frequency within the *wx* locus by means of pollen analysis of the *wx* region and estimated it as 27.3cM/kb, which is about 10 times higher than 250-300 kb/cM for the rice genome on average. Fu et al. (2001) found that an unusually high recombination frequency of the *bronze* locus of maize is associated with a gene-rich region. Duret et al. (2000) observed that regions with a high recombination rate in *Caenorhabditis elegans* preferentially contain class II elements rather than class I elements. The region surrounding the *wx* locus appears to possess both of these properties, high gene density and a higher number of class II elements, found to be associated with a high

recombination frequency in other systems. Therefore, it will be interesting to analyze the recombination frequency in the region examined in this study.

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